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Hepatitis A Virus Vaccine, Strain HM-175, FI-2, Lot No.1 of Jan 89 (5 ml Inactivated with 0.05% Formalin, Adsorbed with Alum containing preservative: 0.375% phenoxyethanol) was satisfactorily (Final Container) safety tested in accordance with the guidelines established by the FDA for live and inactivated vaccines as stipulated in 21 CFR, Parts 610.11 and 610.12. All testing procedures were carried out following Good Laboratory Practices (GLP) regulations (21 CFR, Part 58).

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SAFETY TESTING OF DENGUE-1 AND DENGUE-3 SEEDS FOR HUMAN CHALLENGES, UNATTENUATED; HEPATITIS A VIRUS, STRAIN HM-175

PHASE REPORT

LOUIS POTASH

March 21, 1989

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

CONTRACT NO. DAMD17-86-C-6188

Flow Laboratories, Inc.
McLean, Virginia 22102

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the Guide for the Care and Use of Laboratory Animals prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHHS, PHS, NIH Publications No. 85-23, Revised 1985).

TABLE OF CONTENTS

I. INTRODUCTION

The accompanying protocol is a description of the final container safety testing of a hepatitis A virus vaccine designated as:

Hepatitis A Virus Vaccine, Strain HM-175
FI-2, LOT No. 1, Jan 89
5 ml Inactivated with 0.05% Formalin
Adsorbed with Alum
Preservative: 0.375% phenoxyethanol

Utilizing the testing procedures herein described, this fluid is considered to have passed satisfactorily the tests for Microbial Sterility and General Safety. The detailed records leading to the preparation of this Final Container Vaccine and subsequent safety testing may be found in the laboratory notebooks located at:

The Walter Reed Army Institute of Research (WRAIR), Bldg. 501, Washington, DC 20307-5100 - (Dr. Ken Eckels)

The Experimental Virus Vaccine Production & Testing Laboratory - Suite #500 - Flow Laboratories, Inc., McLean, VA - (Dr. Louis Potash)

In conducting the tests described in this report, the investigator(s) adhered to the Good Laboratory Practices (GLP) regulations (21 CFR, Part 58) and followed the guidelines established by the FDA for live and inactivated vaccines as found in 21 CFR, Parts 610.11 and 610.12, April 1, 1988. The procedures employed are detailed in the following SOPs and recorded on the indicated VVPL Forms:

SOP No.:	400.002 -	Issued	25 Feb 1980,	Revised	8 Feb 1989
	500.009 -	"	23 Feb 1981,	"	3 Mar 1986
	500.013 -	"	14 Dec 1988		
VVPL FORM	#001 -	Issued	25 Feb 1981,	Revised	10 Oct 1988
	#019 -	"	8 Oct 1984		
	#024 -	"	14 Dec 1988		

II. SYNOPSIS

A.	Virus Strain	Hepatitis A, Strain HM-175
B.	Pool Designation	Vaccine FI-2, Lot No. 1, Jan 89 5 ml Inactivatd with 0.05% Formalin Adsorbed with Alum Preservative: 0.375% phenoxyethanol
C.	Treatment/Handling	Stored at 2°-8°C. Manually shaken prior to sampling.

D. Final Product Container Testing (5 x 5 ml vials)

1.	Microbial Sterility: 100 ml volumes of Fluid Thioglycollate & Tryptic Soya Broth Media		No Growth
2.	General Safety:		
	a. Mice - IP	(2 x 0.5 ml)	Satisfactory
	b. Guinea Pigs - IP	(2 x 5.0 ml)	Satisfactory

III. DETAILED SUMMARY RELATING TO THE FINAL CONTAINER TESTING OF A FORMALIN-INACTIVATED, ALUM-ADSORBED HEPATITIS A VIRUS VACCINE, STRAIN HM-175, FI-2 LOT NO. 1.

A. Inoculum

On January 31, 1989, the following Final Container Product was obtained from Dr. K. Eckels, Contracting Officer's Repsresentative, at the Walter Reed Army Institute of Research (WRAIR), Bldg. 501, Washington, DC 20307-5100.

> Hepatitis A Virus Vaccine, Strain HM-175
> FI-2, LOT No. 1, Jan 89
> 5 ml Inactivated with 0.05% Formalin
> Adsorbed with Alum,
> Preservative: 0.375% phenoxyethanol
> Caution: New Drug Limited by
> (USA) Law to Investigational Use
> Dept. Biol. Rsch. WRAIR Wash DC: 5 x 5 ml vials

The vials were stored in a refrigerator (2° - $8^{\circ}C$).

B. Final Product Testing and Results

1. Microbial Sterility

In an effort to overcome any bacteriocidal or bacteriostatic affect of the preservative present in the vaccine, the assay for microbial sterility was based on the use of a 1 ml inoculum into 100 ml volumes of media. Initially, each vial was manually shaken and then, with the use of individual 1 ml tuberculin syringes w/needles, each of 5 bottles of Fluid Thioglycollate Medium (FTM, LOT VVPL-007) and each of 5 bottles of Tryptic Soya Broth (TSB, LOT VVPL-007) were inoculated with 1 ml amounts of the vaccine. Five bottles of each medium were included as uninoculated controls. All cultures were well shaken and incubated at $31^{\circ}C$ ($\pm$ $1^{\circ}C$) and at 22° ($\pm$ $2^{\circ}C$), respectively, for 21 days with periodic observation for growth. No growth was observed in any of the cultures. The results are summarized in Table I.

2. General Safety Test

Initially, a master pool of the vaccine was prepared by pooling 2.5 ml amounts from each of the 5 available vials. Each of 2 overtly healthy CD-1 mice (less than 20 grams each) and each of 2 overtly healthy guinea pigs (Hartley strain, virus free - less than 400 grams each) were inoculated intraperitoneally with 0.5 ml amd 5.0 ml, respectively, of the master pool. Two additional animals of each species were included as uninoculated controls. All animals were weighed prior to inoculation and on day 7 post inoculation. All animals were observed daily over this 7 day period for deaths and/or signs of illness or distress - none were recorded. All animals remained healthy and all exhibited weight gains. The results of these General Safety tests are summarized in Table II.

Table I. Microbial Sterility Test Results on the Hepatitis A Virus Vaccine, Strain HM175, Vaccine FI-2, Lot No. 1, Jan 89
Inactivated with 0.05% Formalin, Adsorbed with Alum
Preservative: 0.375% phenoxyethanol

Culture Medium	No.	Vol. per culture (ml)	Temperature	Date On Test	Date Off Test	Results
Fluid Thioglycollate (FTM) LOT #VVPL-007	5	-----	31°C (±1°C)	2/24/89	3/17/89	No Growth
Alum Vaccine	5	1.0		2/24/89	3/17/89	No Growth
Tryptone Soya Broth (TSB) LOT #VVPL-007	5	-----	22°C (±2°C)	2/24/89	3/17/89	No Growth
Alum Vaccine	5	1.0		2/24/89	3/17/89	No Growth

Table II. General Safety Test Results on the Hepatitis A Virus Inactivated with 0.05% Formalin, Adsorbed with Alum Preservative: 0.375% phenoxyethanol

Animal Species	Inoculum	Vol. (ml)	Tag #	Weight in Grams Day 0	Weight in Grams Day 7	Weight Gain/ (Loss) in Grams
Mice	Vaccine	0.5	339	18.8	25.5	6.7
			340	16.5	27.5	11.0
	None	—	337	19.2	26.1	6.9
			338	17.0	28.5	11.5
Guinea Pigs	Vaccine	5.0	503	371.1	479.5	108.4
			504	393.0	469.2	76.2
	None	—	501	378.1	435.7	57.6
			502	403.0	480.2	77.2

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